Thursday, 4 January, 2012
27633 IT and Health

Genotype and Phenotype

Outline for this afternoon

- SNP
- Strand orientation and complementary bases
- Genotype
- Homozygotes and heterozygotes
- SNP genotyping
- Phenotype
- Associating genotype and phenotype

- Case story: Genotype and phenotype of an ancient genome

- Main exercise: Genotype to phenotype

SNP

Sequence variations

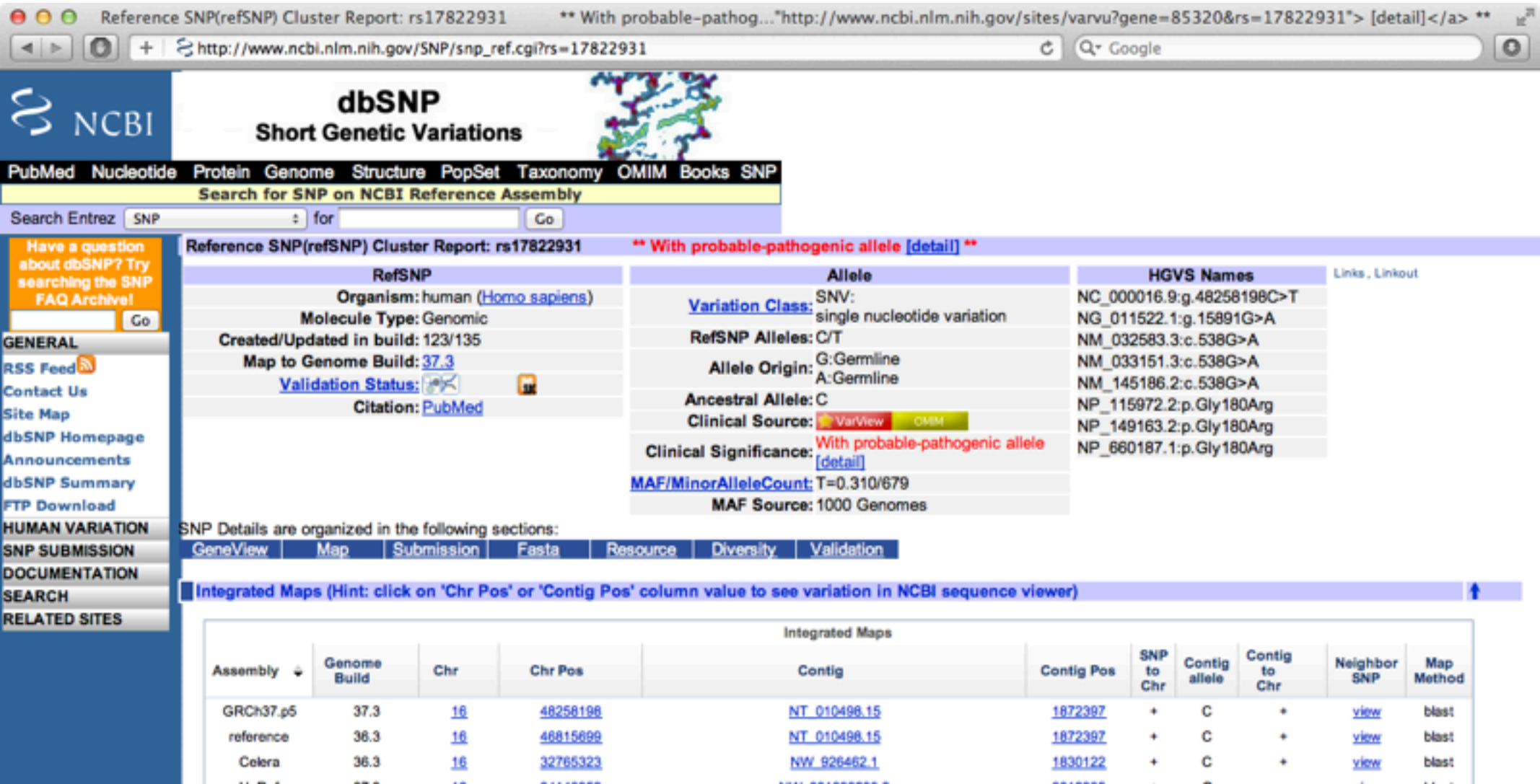
- Single nucleotide polymorphisms
- Insertions/deletions
- Copy number variations (large: >1kb)
- Variable (short) number tandem repeats

Single Nucleotide Polymorphisms (SNPs)

- A single nucleotide (A,T,C,G) DNA sequence alteration
  - … **ACGGCTAA** …
  - … **ATGGCTAA** …
- It must occur in at least 1% of the population
- 10–30 million SNPs
- The most common kind of human genetic variations
- Occur every 100-300 bases along the 3-billion-base human genome
- Evolutionary stable

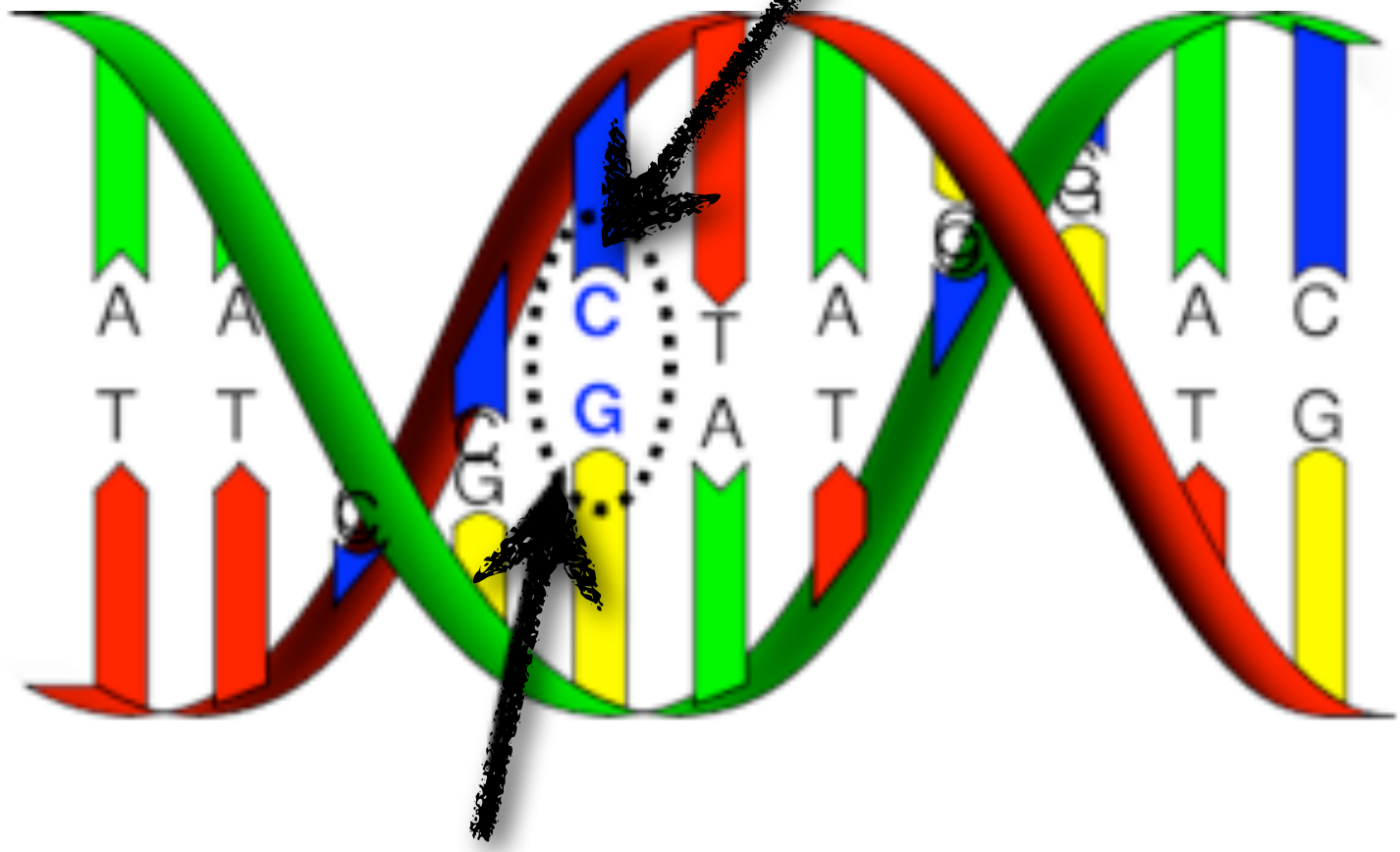
SNP database: dbSNP

- rs numbers
- chromosome and positions (ncbi36 vs GRCh37)



DNA is double-stranded

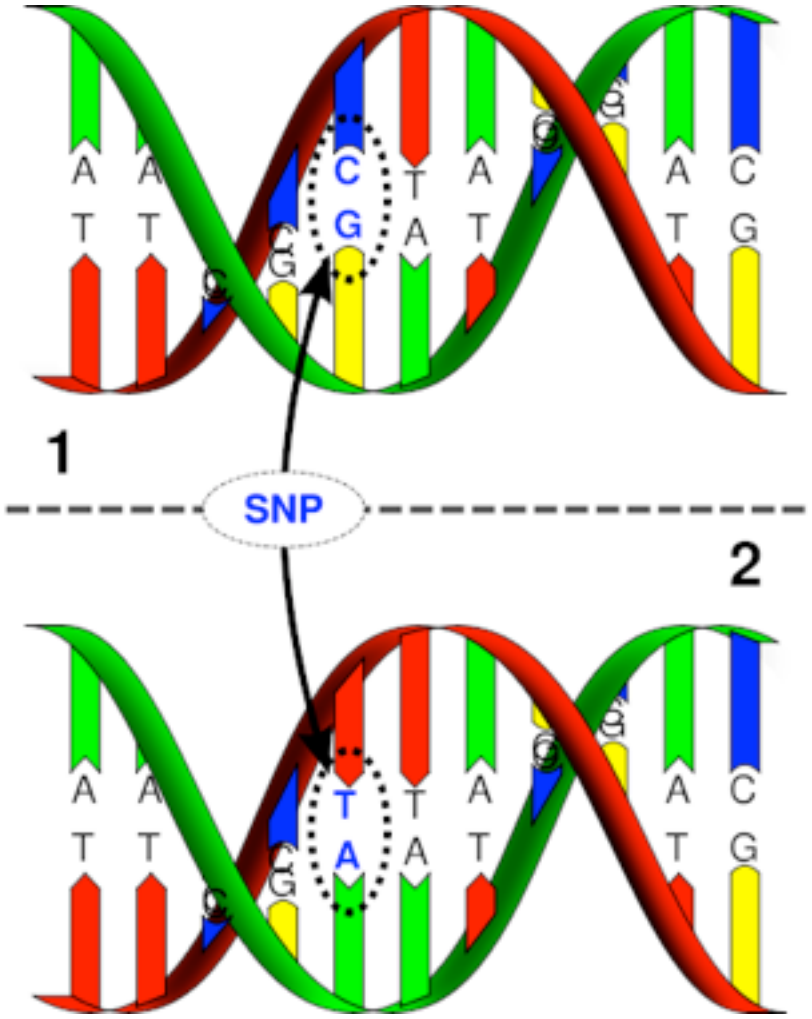
....and "C" on the other



"G" on one strand...

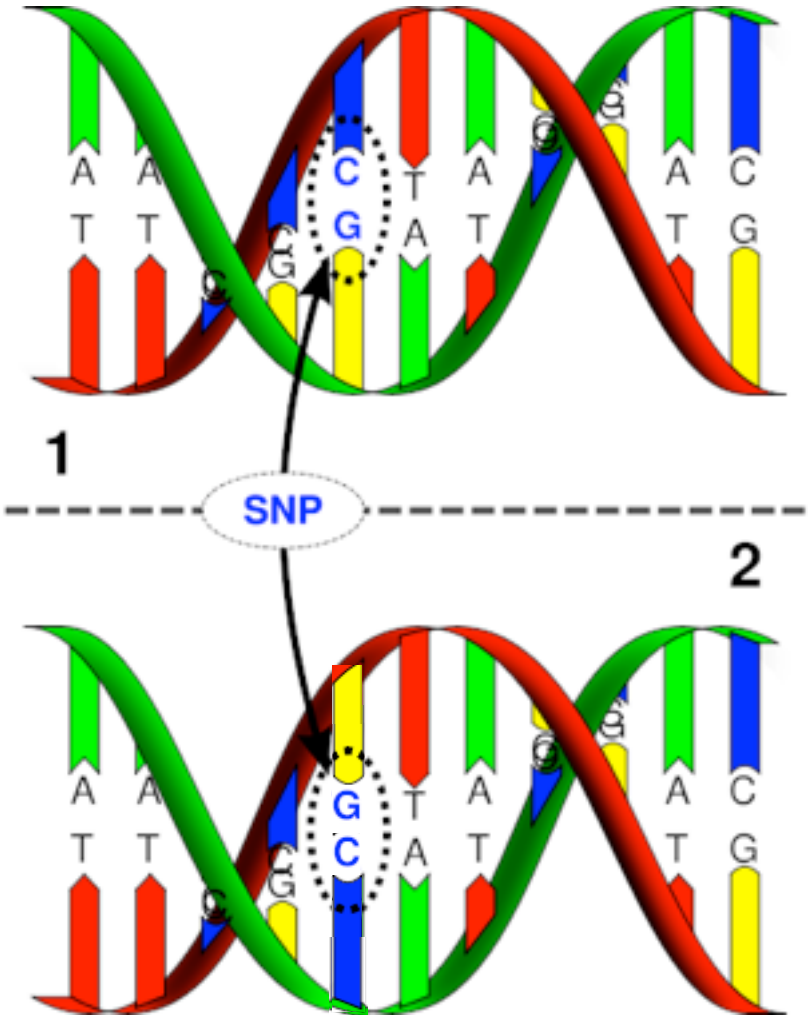
SNP A

"C" or "T" on the red strand
"G" or "A" on the green strand



SNP B

"C" or "G" on the red strand
"G" or "C" on the green strand



Genotype of an individual for a given SNP

One copy of rs17822931 from the father and one copy from the mother

Copy 1:

5′...GGCCTGAGT...3′ (+)

3′...CCGGACTCA...5′ (−)

Copy 2:

5′...GGCCCGAGT...3′ (+)

3′...CCGGGCTCA...5′ (−)

Genotype for rs17822931 on plus strand:

T;C (T;C) C,T T,C (T,C)

(C;T) CT C;T rs17822931(T;C) TC

rs17822931(C;T)

Genotype for rs17822931 on minus strand:

A;G (A;G) C,T A,G (A,G)

(G;A) GA G;A rs17822931(A;G) AG

rs17822931(G;A)

exercise

	rs4788084	rs17822931	rs73546424		
Copy 1:	**5′...TCCCCTGGG...GGCCTGAGT...TGCATGTGA...**			**3′**	**(+)**
	3′...AGGGGACCC...CCGGACTCA...ACGTACACT...			5′	(−)
Copy 2:	**5′...TCCCCTGGG...GGCCCGAGT...TGCAAGTGA...**			**3′**	**(+)**
	3′...AGGGGACCC...CCGGGCTCA...ACGTTCACT...			5′	(−)

	rs4788084 dbSNP orientation: minus	rs17822931 dbSNP orientation: plus	rs73546424 dbSNP orientation: plus
genotype on “plus strand”			
genotype on “minus strand”			
genotype on “dbSNP strand”			

exercise

rs4788084 rs17822931 rs73546424

Copy 1:

5′...TCCCCTGGG...GGCCTGAGT...TGCATGTGA... 3′ (+)

3′...AGGGGACCC...CCGGACTCA...ACGTACACT... 5′ (−)

Copy 2:

5′...TCCCCTGGG...GGCCCGAGT...TGCAAGTGA... 3′ (+)

3′...AGGGGACCC...CCGGGCTCA...ACGTTCACT... 5′ (−)

	rs4788084 dbSNP orientation: minus	rs17822931 dbSNP orientation: plus	rs73546424 dbSNP orientation: plus
genotype on “plus strand”	C;C	T;C or alphabetically C;T	T;A or alphabetically A;T
genotype on “minus strand”	G;G	A;G	A;T
genotype on “dbSNP strand”	G;G	T;C or alphabetically C;T	T;A or alphabetically A;T

Homozygotes and heterozygotes

Homozygous:

Genotype consisting of two identical alleles at a given locus
(For a SNP: the same base at both copies, eg. C;C or A;A)

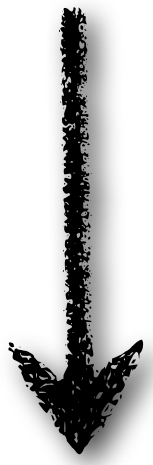
Heterozygous:

Genotype consisting of two different alleles at a locus
(For a SNP: the same base at both copies, eg. A;C)

exercise

	rs4788084	rs17822931	rs73546424	
Copy 1:	**5′...TCCCCTGGG...GGCCTGAGT...TGCATGTGA...**	**3′**	**(+)**
	3′...AGGGGACCC...CCGGACTCA...ACGTACACT...	5′	(−)
Copy 2:	**5′...TCCCCTGGG...GGCCCGAGT...TGCAAGTGA...**	**3′**	**(+)**
	3′...AGGGGACCC...CCGGGCTCA...ACGTTCACT...	5′	(−)

heterozygous
or
homozygous
?



heterozygous
or
homozygous
?

heterozygous
or
homozygous
?

exercise

rs4788084 rs17822931 rs73546424

Copy 1:

5′...TCCCCTGGG...GGCCTGAGT...TGCATGTGA... 3′ (+)

3′...AGGGGACCC...CCGGACTCA...ACGTACACT... 5′ (−)

Copy 2:

5′...TCCCCTGGG...GGCCCGAGT...TGCAAGTGA... 3′ (+)

3′...AGGGGACCC...CCGGGCTCA...ACGTTCACT... 5′ (−)

	rs4788084 dbSNP orientation: minus	rs17822931 dbSNP orientation: plus	rs73546424 dbSNP orientation: plus
genotype on “plus strand”	C;C	C;T	A;T
genotype on “minus strand”	G;G	A;G	A;T
genotype on “dbSNP strand”	G;G	C;T	A;T
zygosity	homozygous	heterozygous	heterozygous

Obtaining the SNP genotypes (genotyping)

SNP arrays

0.5 – 1 million SNPs

Affymetrix SNP 6.0

Affymetrix SNP 5.0

Affymetrix GeneChip Human Mapping 250K

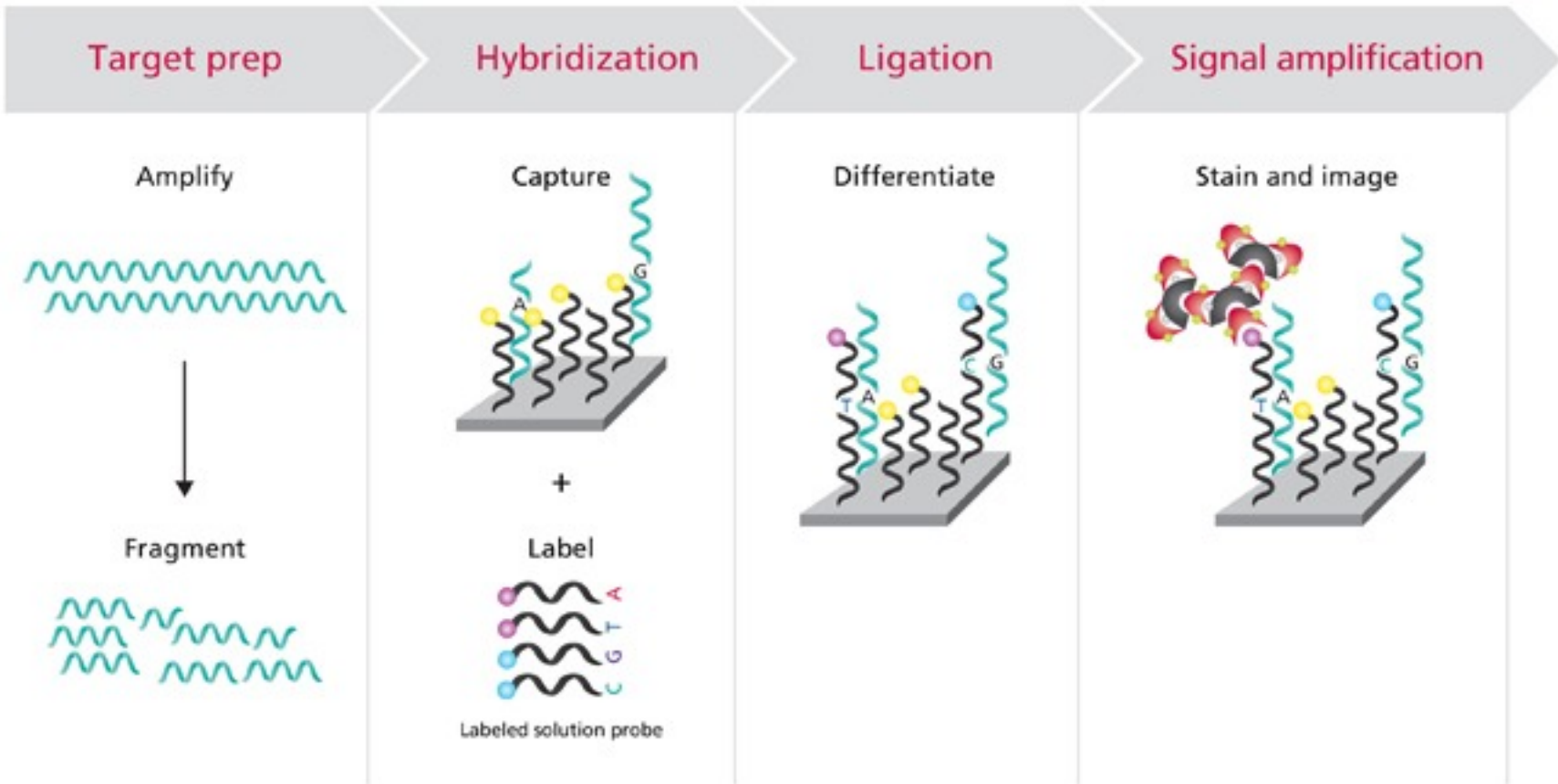
Affymetrix Axiom GW

Illumina Human Hap 650v3

Illumina Human Hap 550v3

Illumina Human Hap 300v3

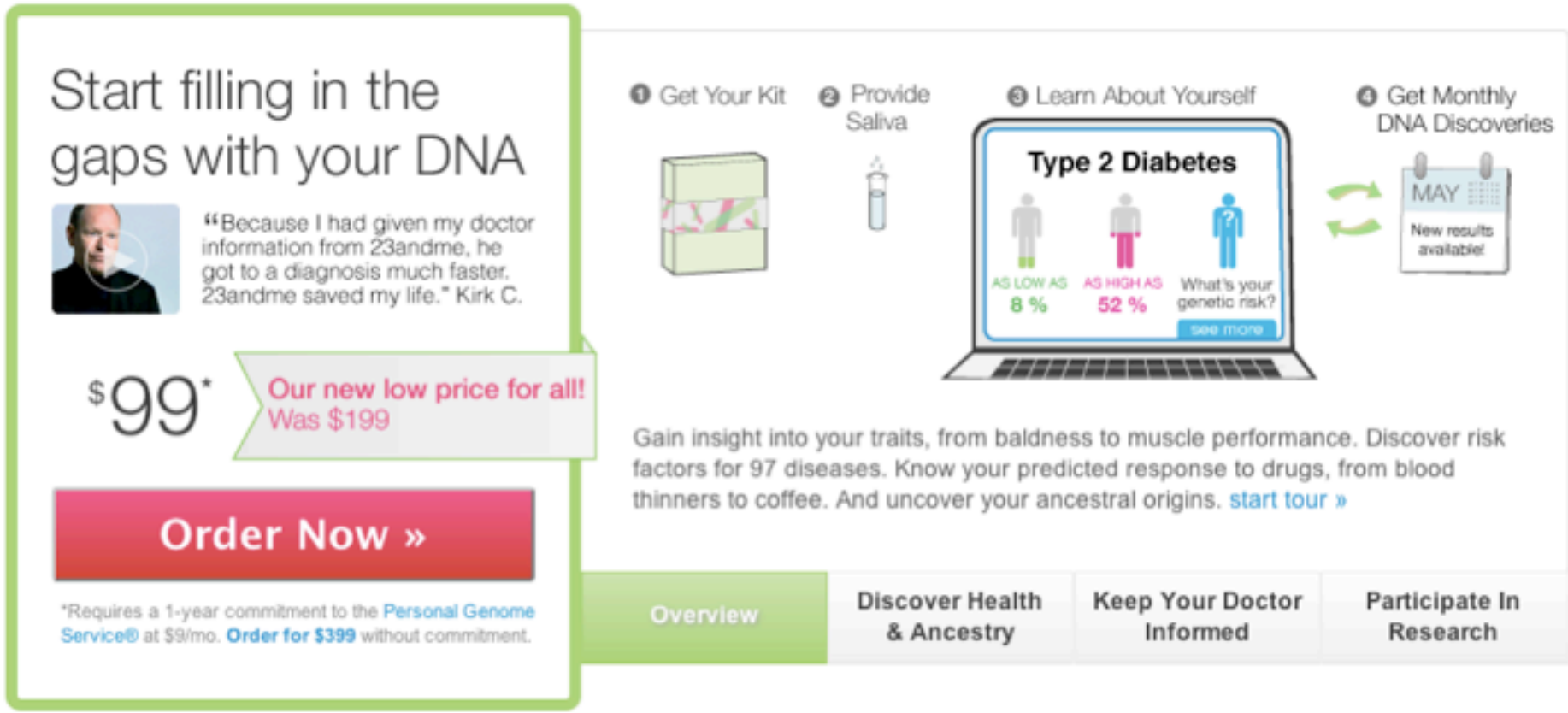
SNP arrays


Target prep
Hybridization
Ligation
Signal amplification
Amplify
Fragment
Capture
+
Label
Labeled solution probe
Differentiate
Stain and image

Commercial genotyping companies

- 23andme
- deCODEme
- Navigenics

~ 1 million SNPs



What is a phenotype?

An observable characteristics or trait

obesity

eye color

height

A method for connecting genotypes and phenotypes

Genome-wide association studies (GWAS)

Cases vs controls

- Obtain DNA from a disease group (e.g. asthma) and a control group
- Obtain genotypes
- Identify variants that are significantly more common among cases than controls
- Those SNPs are associated with the disease (in this study)
- Not necessarily causal

GWAS results: p-values

Example of GWAS results (asthma)

Manhattan plot displays all SNPs on x-axis (order by genomic location), and -$\log_{10}$ of p-values on y-axis.

Figure 2. Manhattan Plot of the Results from the Combined Subjects of European Ancestry Who Had Asthma.

The $-\log_{10}$ P values are plotted against the physical distance. Only the two loci at chromosome 1q31 and 17q21 were significantly associated with asthma after Bonferroni correction. Individual chromosome labels are indicated in white within the Manhattan plot.

Variants of *DENND1B* Associated with Asthma in Children

Patrick M.A. Sleiman, Ph.D., James Flory, Ph.D., Marcin Imielinski, M.D., Ph.D., Jonathan P. Bradfield, B.S., Kiran Annaiah, M.Sc., Saffron A.G. Willis-Owen, Ph.D., Kai Wang, Ph.D., Nicholas M. Rafaels, M.S., Sven Michel, Ph.D., Klaus Bonnelykke, M.D., Ph.D., Haitao Zhang, Ph.D., Cecilia E. Kim, B.A., Edward C. Frackelton, B.A., Joseph T. Glessner, M.Sc., Cuiping Hou, M.Sc., F. George Otieno, M.Sc., Erin Santa, B.A., Kelly Thomas, B.A., Ryan M. Smith, B.A., Wendy R. Glaberson, B.A., Maria Garris, B.A., Rosetta M. Chiavacci, B.S.N., Terri H. Beaty, Ph.D., Ingo Ruczinski, Ph.D., Jordan M. Orange, M.D., Ph.D., Julian Allen, M.D., Jonathan M. Spergel, M.D., Ph.D., Robert Grundmeier, M.D., Ph.D., Rasika A. Mathias, Sc.D., Jason D. Christie, M.D., Erika von Mutius, M.D., William O.C. Cookson, M.D., Michael Kabesch, M.D., Miriam F. Moffatt, Ph.D., Michael M. Grunstein, M.D., Ph.D., Kathleen C. Barnes, Ph.D., Marcella Devoto, Ph.D., Mark Magnusson, M.D., Hongzhe Li, Ph.D., Struan F.A. Grant, Ph.D., Hans Bisgaard, M.D., and Hakon Hakonarson, M.D., Ph.D.

N Engl J Med 2010; 362:36-44 | January 7, 2010

Online phenotype association resources

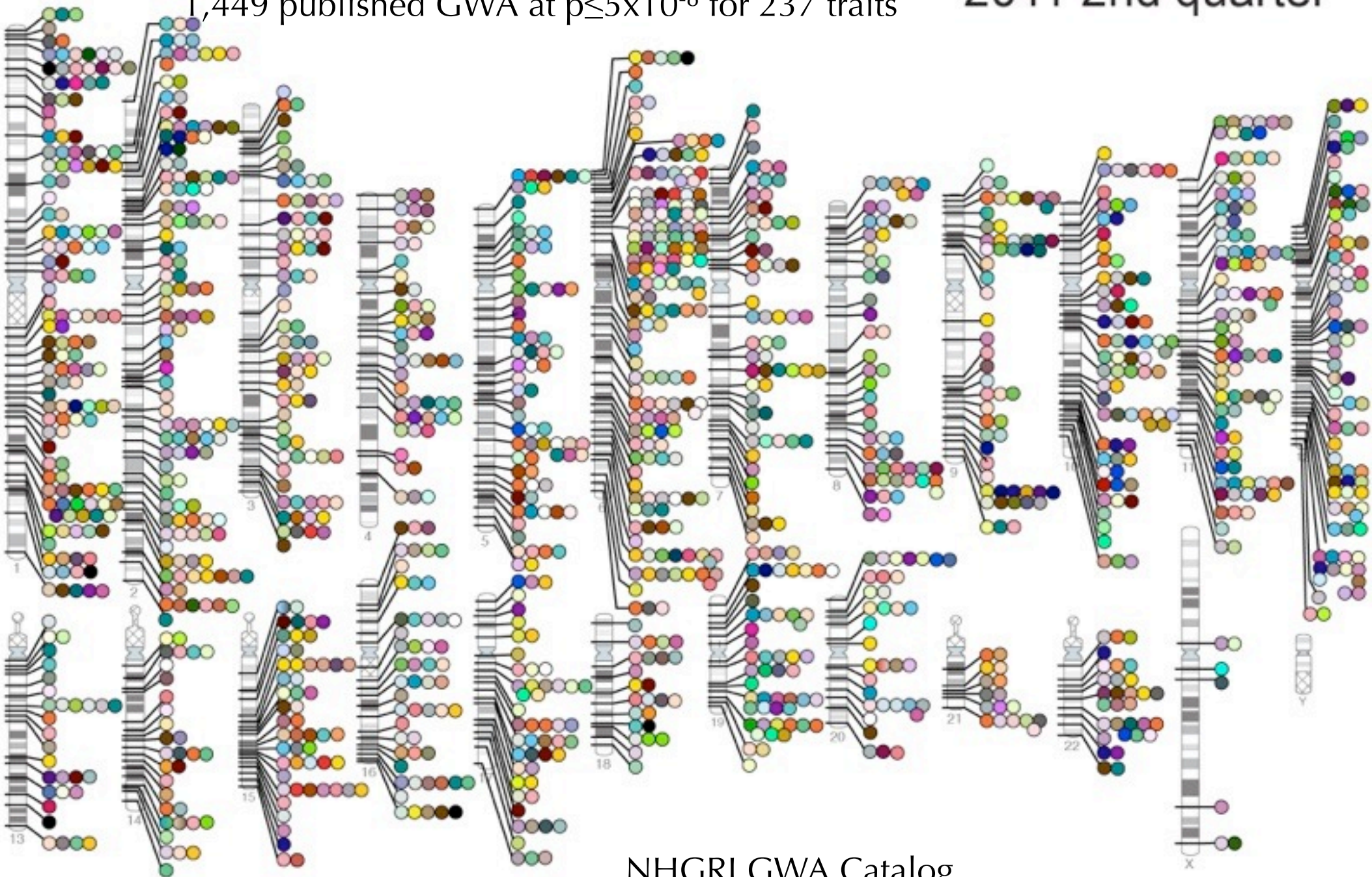
- NHGRI's Catalog of Published GWAS:

  http://www.genome.gov/GWAStudies/

- SNPedia:

  http://www.snpedia.com

Published Genome-Wide Associations through 06/2011,
1,449 published GWA at $p \leq 5 \times 10^{-8}$ for 237 traits



NHGRI GWA Catalog
www.genome.gov/GWAStudies


Abdominal aortic aneurysm
Acute lymphoblastic leukemia
Adhesion molecules
Adiponectin levels
Age-related macular degeneration
AIDS progression
Alcohol dependence
Alopecia areata
Alzheimer disease
Amyloid A levels
Amyotrophic lateral sclerosis
Angiotensin-converting enzyme activity
Ankylosing spondylitis
Arterial stiffness
Asparagus anosmia
Asthma
Atherosclerosis in HIV
Atrial fibrillation
Attention deficit hyperactivity disorder
Autism
Basal cell cancer
Behcet's disease
Bipolar disorder
Biliary atresia
Bilirubin
Bitter taste response
Birth weight
Bladder cancer
Bleomycin sensitivity
Blond or brown hair
Blood pressure
Blue or green eyes
BMI, waist circumference
Bone density
Breast cancer
C-reactive protein
Calcium levels
Cardiac structure/function
Cardiovascular risk factors
Carnitine levels
Carotenoid/tocopherol levels
Celiac disease
Celiac disease and rheumatoid arthritis
Cerebral atrophy measures
Chronic lymphocytic leukemia
Chronic myeloid leukemia
Cleft lip/palate
Coffee consumption
Cognitive function
Conduct disorder
Colorectal cancer
Corneal thickness
Coronary disease
Creutzfeldt-Jakob disease
Crohn's disease
Crohn's disease and celiac disease
Cutaneous nevi
Cystic fibrosis severity
Dermatitis
DHEA-s levels
Diabetic retinopathy
Dilated cardiomyopathy
Drug-induced liver injury
Drug-induced liver injury (amoxicillin-clavulanate)
Endometrial cancer
Endometriosis
Eosinophil count
Eosinophilic esophagitis
Erectile dysfunction and prostate cancer treatment
Erythrocyte parameters
Esophageal cancer
Essential tremor
Exfoliation glaucoma
Eye color traits
F cell distribution
Fibrinogen levels
Folate pathway vitamins
Follicular lymphoma
Fuch's corneal dystrophy
Freckles and burning
Gallstones
Gastric cancer
Glioma
Glycemic traits
Hair color
Hair morphology
Handedness in dyslexia
HDL cholesterol
Heart failure
Heart rate
Height
Hemostasis parameters
Hepatic steatosis
Hepatitis
Hepatocellular carcinoma
Hirschsprung's disease
HIV-1 control
Hodgkin's lymphoma
Homocysteine levels
Hypospadias
Idiopathic pulmonary fibrosis
IFN-related cytopeni
IgA levels
IgE levels
Inflammatory bowel disease
Insulin-like growth factors
Intracranial aneurysm
Iris color
Iron status markers
Ischemic stroke
Juvenile idiopathic arthritis
Keloid
Kidney stones
LDL cholesterol
Leprosy
Leptin receptor levels
Liver enzymes
Longevity
LP (a) levels
LpPLA(2) activity and mass
Lung cancer
Magnesium levels
Major mood disorders
Malaria
Male pattern baldness
Mammographic density
Matrix metalloproteinase levels
MCP-1
Melanoma
Menarche & menopause
Meningococcal disease
Metabolic syndrome
Migraine
Moyamoya disease
Multiple sclerosis
Myeloproliferative neoplasms
Myopia (pathological)
N-glycan levels
Narcolepsy
Nasopharyngeal cancer
Natriuretic peptide levels
Neuroblastoma
Nicotine dependence
Obesity
Open angle glaucoma
Open personality
Optic disc parameters
Osteoarthritis
Osteoporosis
Otosclerosis
Other metabolic traits
Ovarian cancer
Pancreatic cancer
Pain
Paget's disease
Panic disorder
Parkinson's disease
Periodontitis
Peripheral arterial disease
Personality dimensions
Phosphatidylcholine levels
Phosphorus levels
Photic sneeze
Phytosterol levels
Platelet count
Polycystic ovary syndrome
Primary biliary cirrhosis
Primary sclerosing cholangitis
PR interval
Progranulin levels
Progressive supranuclear palsy
Prostate cancer
Protein levels
PSA levels
Psoriasis
Psoriatic arthritis
Pulmonary funct. COPD
QRS interval
QT interval
Quantitative traits
Recombination rate
Red vs.non-red hair
Refractive error
Renal cell carcinoma
Renal function
Response to antidepressants
Response to antipsychotic therapy
Response to carbamazepine
Response to clopidogrel therapy
Response to hepatitis C treat
Response to interferon beta therapy
Response to metaformin
Response to statin therapy
Restless legs syndrome
Retinal vascular caliber
Rheumatoid arthritis
Ribavirin-induced anemia
Schizophrenia
Serum metabolites
Skin pigmentation
Smoking behavior
Speech perception
Sphingolipid levels
Statin-induced myopathy
Stroke
Sudden cardiac arrest
Suicide attempts
Systemic lupus erythematosus
Systemic sclerosis
T-tau levels
Tau AB1-42 levels
Telomere length
Testicular germ cell tumor
Thyroid cancer
Thyroid volume
Tooth development
Total cholesterol
Triglycerides
Tuberculosis
Type 1 diabetes
Type 2 diabetes
Ulcerative colitis
Urate
Urinary albumin excretion
Urinary metabolites
Uterine fibroids
Venous thromboembolism
Ventricular conduction
Vertical cup-disc ratio
Vitamin B12 levels
Vitamin D insufficiency
Vitiligo
Warfarin dose
Weight
White cell count
White matter hyperintensity
YKL-40 levels

SNPedia

Earwax

rs17822931 determines wet vs dry earwax [PMID 16444273]

This can also be used to distinguish asian ancestry.

NCBI coffeebreak introduction

Category: Is a medical condition

Navigation

SNPedia
FAQ
Blog
Recent changes
Random page

Toolbox

What links here
Related changes
Special pages
Printable version
Permanent link
Browse properties

Genotype and phenotype of an ancient genome

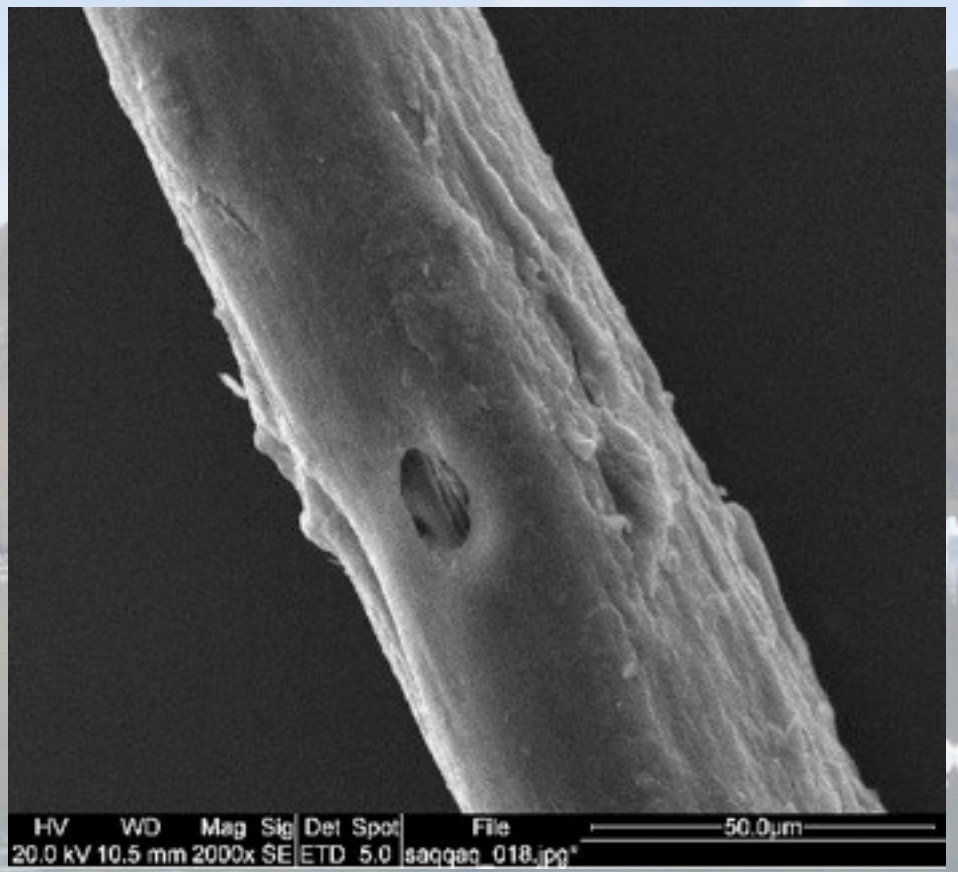
First Ancient Human Genome

The Saqqaq Genome Project

4,000 years
Hair sample from permafrost
DNA extraction <10% contamination
20 x coverage
Started 2009

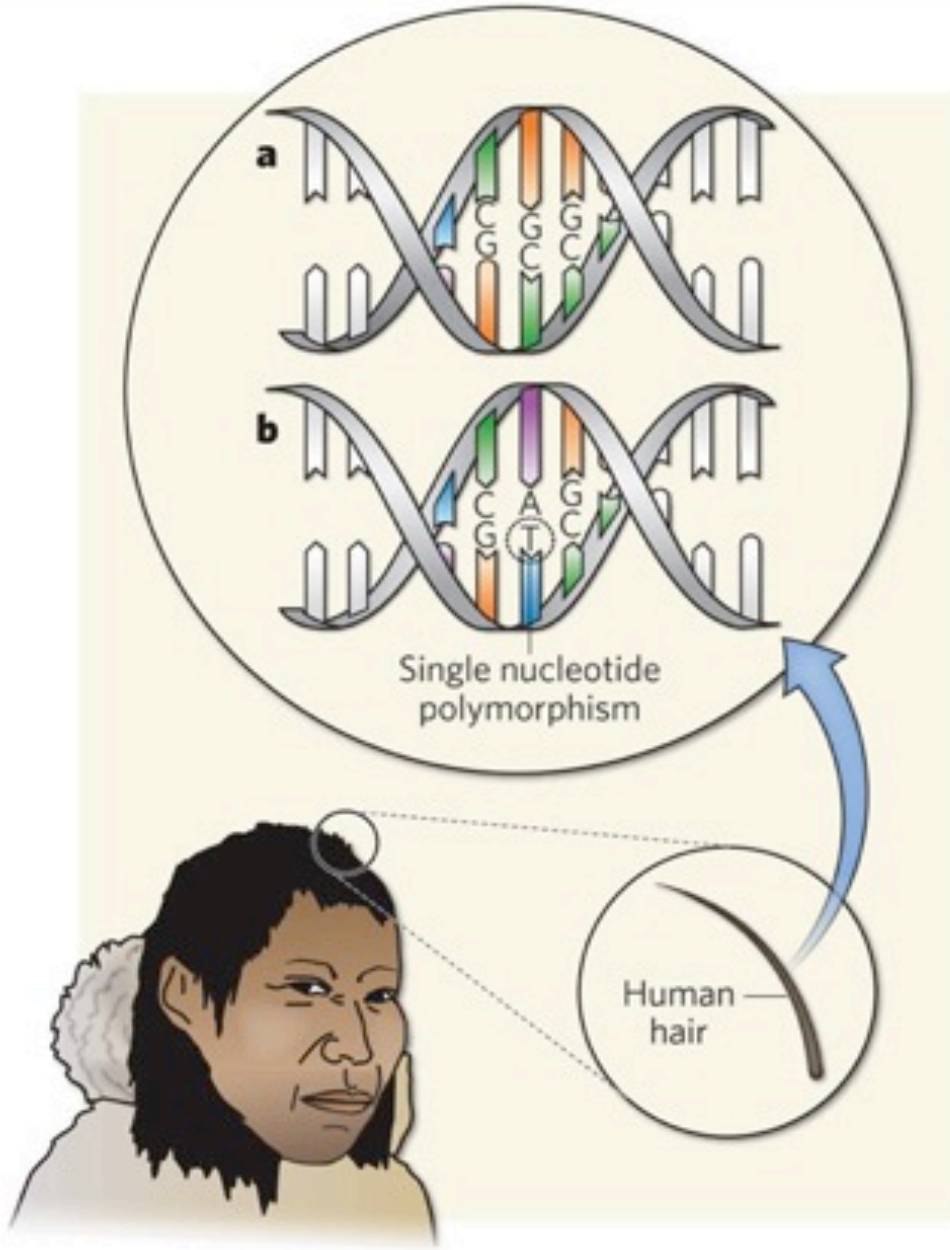
Eske Willerslev

DNA from hair



DNA from hair

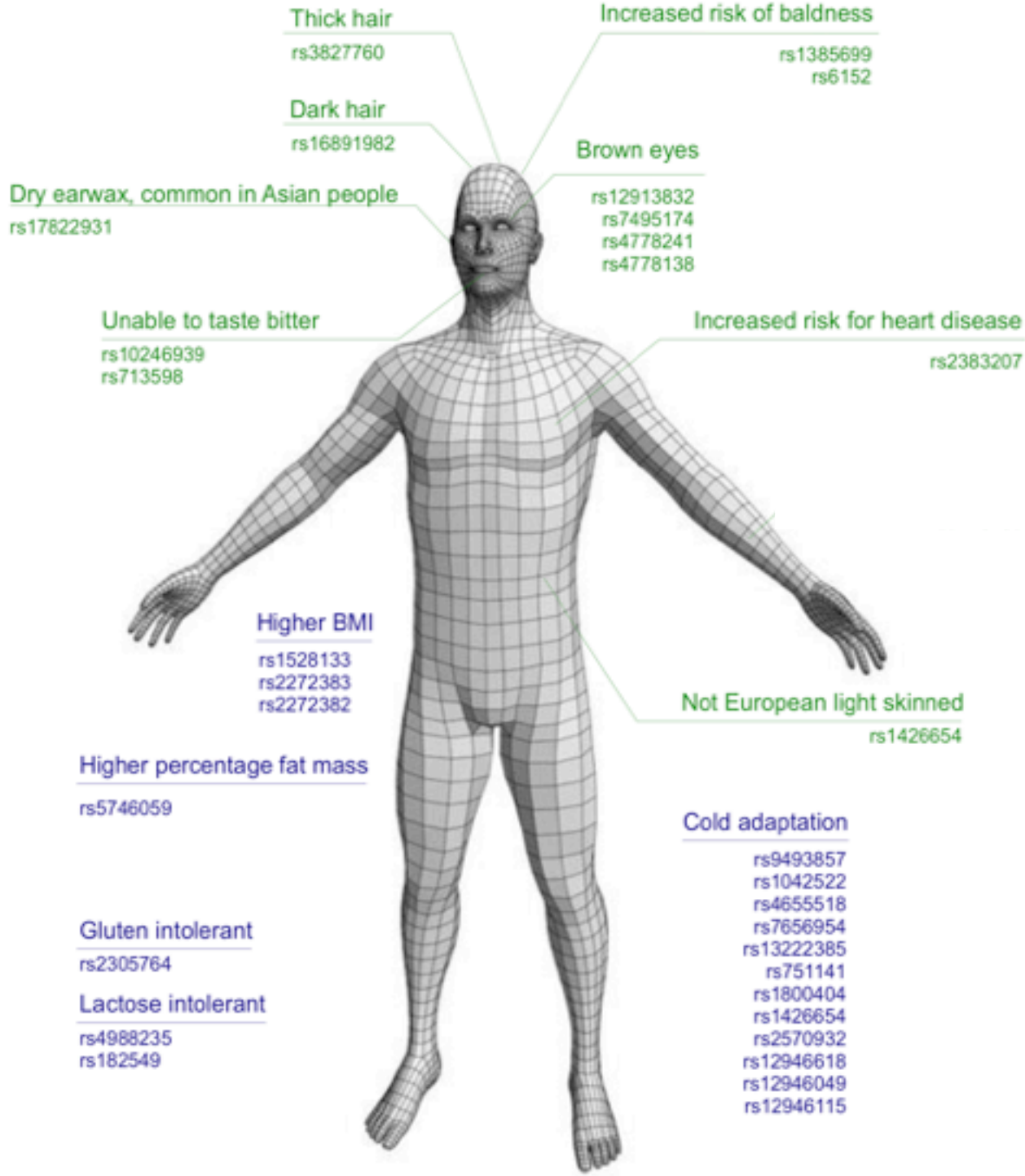
- Hardly any contamination
- Relatively high yield
- Short fragments
- <3 month sequencing
- Post-mortem DNA damage
- Polymerase Phusion



Rasmussen *et al.*[2] have sequenced the genome of a man from the Saqqaq culture, using DNA from hair preserved in permafrost in Greenland. They analysed the genome to find single nucleotide polymorphisms (SNPs) — differences in single DNA base pairs that exist between individual genomes, and that may act as markers of an individual's physical traits. **a**, Here, a short stretch of human DNA is shown that is a marker for normal earwax. **b**, In the analogous DNA from the Saqqaq individual, there is a SNP in which a C in the lower strand has been replaced by a T (C, G, T and A denote the four kinds of DNA base). This SNP shows that the Saqqaq man had dry earwax. Rasmussen and colleagues identified other SNPs indicating that the ancient human had, among other things, brown eyes, non-white skin, thick dark hair and an increased susceptibility to baldness.

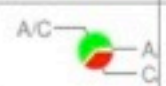
V. WARD, UNIV. AUCKLAND

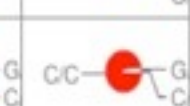
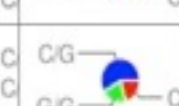
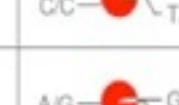
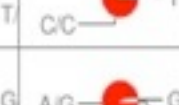
What can we say about his phenotypes?



From genotype to phenotype: how good are we at putting a face to an anonymous individual? - While some traits manifest themselves in a tissue specific manner (highlighted in green), others are more systemic (highlighted in blue). Going from the genetic blueprint to visual appearance, physiological behaviour and medical predispositions is still an open challenge.

Table S14. List of SNP identifiers used for functional assessment. Associations to phenotype were curated from literature, public databases and HGMD Professional. Frequencies from Hapmap Phase-II are represented in pie charts, with the colour of the Saqqaq Allele matching the corresponding genotype in the pie charts. In most cases, the Saqqaq allele is closest to the Asian (Han Chinese and Japanese) populations.

SNP	Reference	1-pp	Saqqaq Allele	HapMap frequencies			
				CEU	*HCB*	*JPT*	*YRI*
rs8176719[1]	66	NA	-/-	NA	NA	NA	NA
rs8176750[2]	67	8.84E-34	G/G	NA	NA	NA	NA
rs12913832[3]	68	5.13E-10	A/A				
rs7495174[3]	68	1.14E-25	A/G				
rs4778241[3]	68	8.24E-06	A/A				
rs1129038[3]	68	8.69E-40	C/C	NA	NA	NA	NA
rs1426654[4]	69	8.78E-12	G/G				
rs1385699[5]	70	8.57E-05	T/T				
rs6152[5]	71	4.12E-21	G/G	NA	NA	NA	NA
rs1528133[6]	63	1.50E-08	A/C				
rs2272383[6]	63	1.42E-27	A/G				
rs2272382[6]	63	8.40E-60	A/G				
rs5746059[7]	62	1.64E-05	A/A				

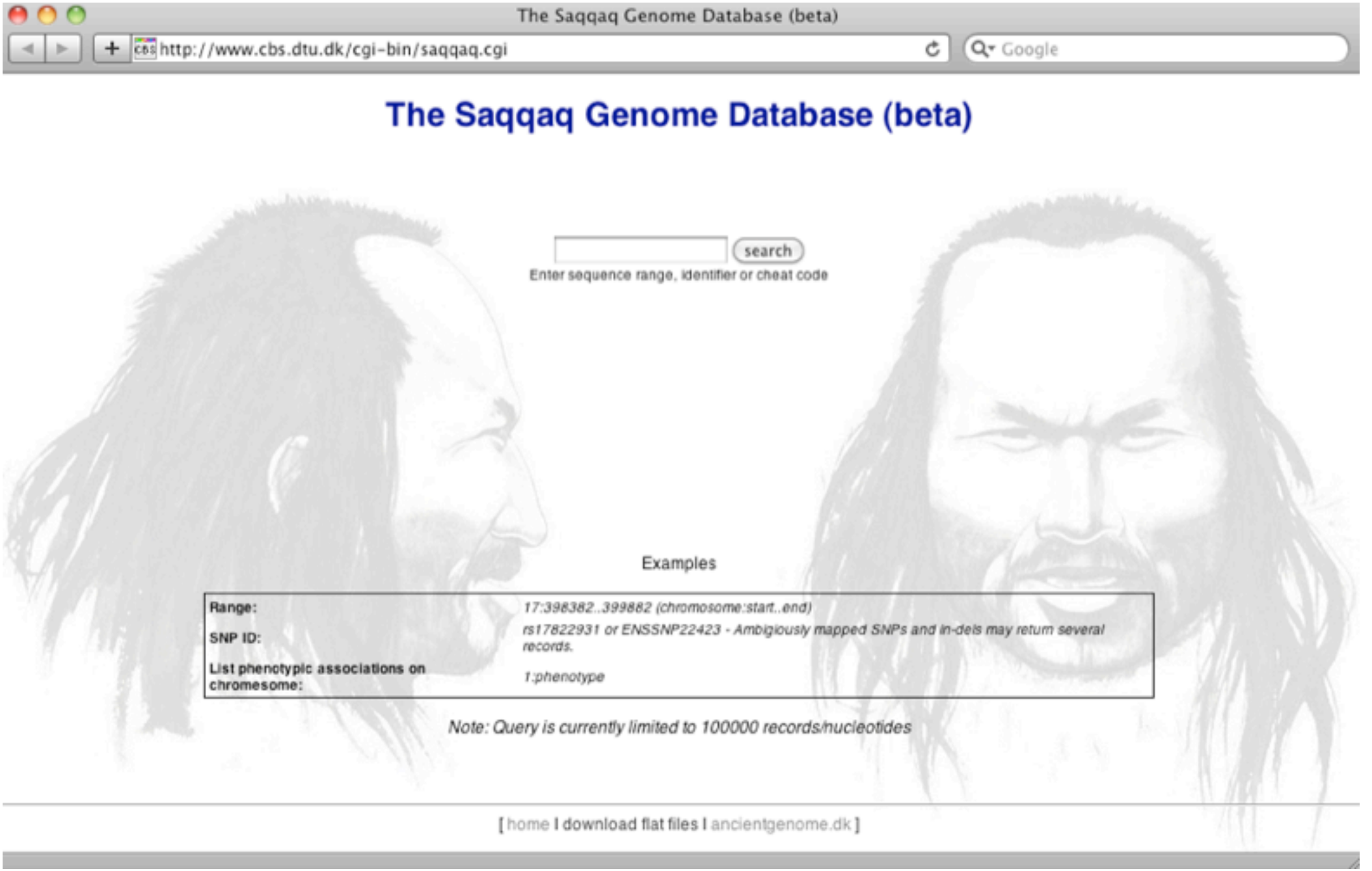
rs17822931[8]	72	8.36E-09	T/T				
rs3827760[9]	73	5.61E-12	C/C				
rs16891982[10]	74	3.87E-13	C/C				
rs1042522[11]	75	2.75E-11	G/G				
rs13222385[12]	59	3.21E-05	A/G				
rs751141[12]	59	2.13E-07	T/T				
rs1800404[12]	59	2.51E-14	A/A				
rs1426654[12]	59	8.78E-12	G/G				
rs2570932[12]	59	0.0015097	C/C				
rs12946618[12]	59	0.0013802	A/A				
rs12946115[12]	59	2.44E-06	C/C				

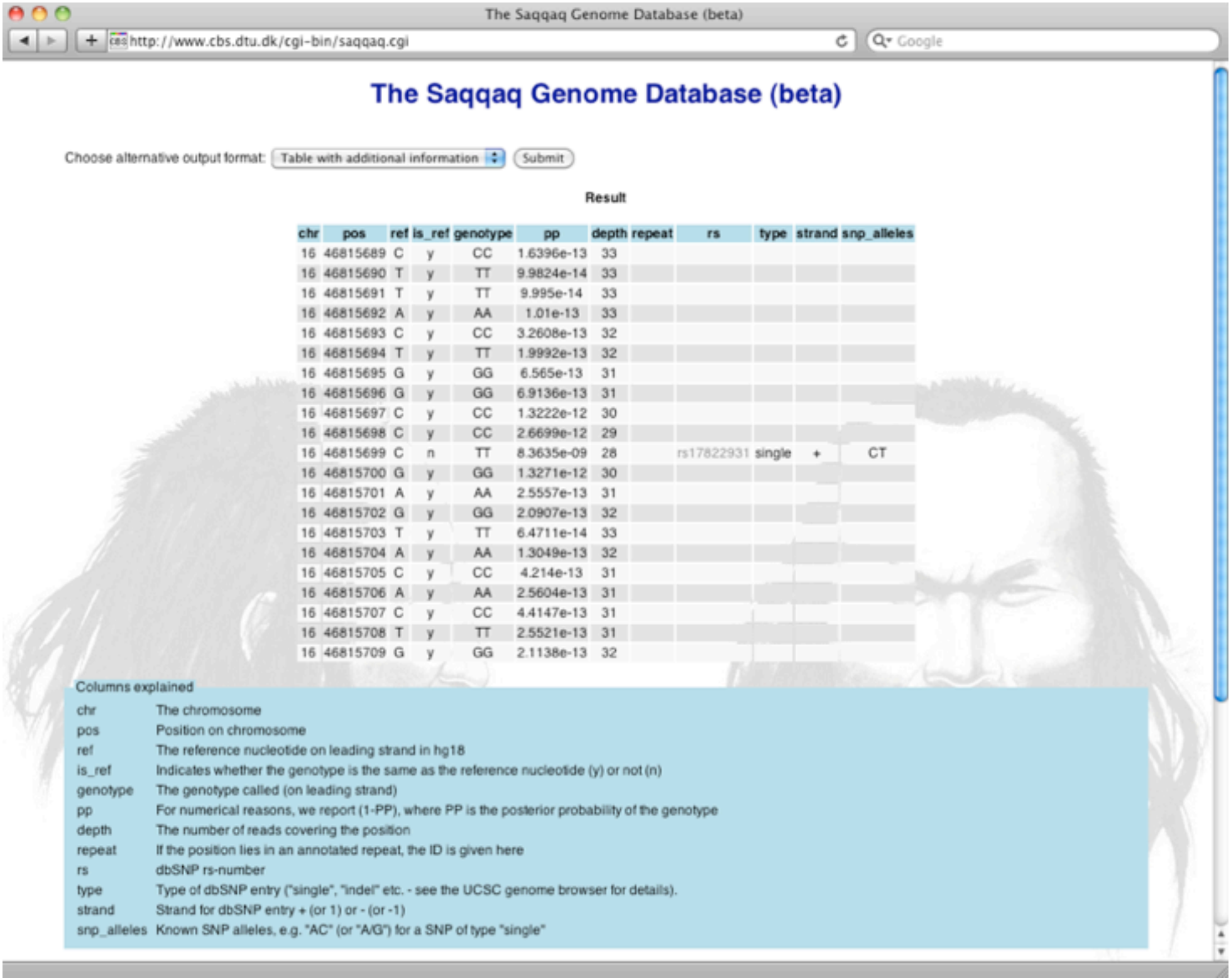
[1]Blood Group: not type O. [2]Blood Group: A1 subtype. [3]Brown eyes. [4]Not European light skinned. [5]Increased risk of baldness. [6]Higher body mass index. [7]Higher percentage fat mass in Caucasian and Chinese samples. [8]Dry earwax, common in Asian people. [9]Thick hair and Shovel shaped upper front teeth. [10]More likely to have black hair (in European cohort study). [11] Cold adaptation: non-synonymous change in TP53. [12] Cold adaptation: Metabolic genes.

The ancient genome database

www.ancientgenome.dk

- Get sequence
- Compare to reference genome (NCBI 36)
- SNP accessions
- Phenotypic associations


The Saqqaq Genome Database (beta)
http://www.cbs.dtu.dk/cgi-bin/saqqaq.cgi
Google
The Saqqaq Genome Database (beta)
search
Enter sequence range, identifier or cheat code
Examples
Range:
17:398382..399882 (chromosome:start..end)
SNP ID:
rs17822931 or ENSSNP22423 - Ambigiously mapped SNPs and in-dels may return several records.
List phenotypic associations on chromesome:
1:phenotype
Note: Query is currently limited to 100000 records/nucleotides
[home I download flat files I ancientgenome.dk]



chr	pos	ref	is_ref	genotype	pp	depth	repeat	rs	type	strand	snp_alleles
16	46815689	C	y	CC	1.6396e-13	33					
16	46815690	T	y	TT	9.9824e-14	33					
16	46815691	T	y	TT	9.995e-14	33					
16	46815692	A	y	AA	1.01e-13	33					
16	46815693	C	y	CC	3.2608e-13	32					
16	46815694	T	y	TT	1.9992e-13	32					
16	46815695	G	y	GG	6.565e-13	31					
16	46815696	G	y	GG	6.9136e-13	31					
16	46815697	C	y	CC	1.3222e-12	30					
16	46815698	C	y	CC	2.6699e-12	29					
16	46815699	C	n	TT	8.3635e-09	28		rs17822931	single	+	CT
16	46815700	G	y	GG	1.3271e-12	30					
16	46815701	A	y	AA	2.5557e-13	31					
16	46815702	G	y	GG	2.0907e-13	32					
16	46815703	T	y	TT	6.4711e-14	33					
16	46815704	A	y	AA	1.3049e-13	32					
16	46815705	C	y	CC	4.214e-13	31					
16	46815706	A	y	AA	2.5604e-13	31					
16	46815707	C	y	CC	4.4147e-13	31					
16	46815708	T	y	TT	2.5521e-13	31					
16	46815709	G	y	GG	2.1138e-13	32					

Columns explained

chr	The chromosome
pos	Position on chromosome
ref	The reference nucleotide on leading strand in hg18
is_ref	Indicates whether the genotype is the same as the reference nucleotide (y) or not (n)
genotype	The genotype called (on leading strand)
pp	For numerical reasons, we report (1-PP), where PP is the posterior probability of the genotype
depth	The number of reads covering the position
repeat	If the position lies in an annotated repeat, the ID is given here
rs	dbSNP rs-number
type	Type of dbSNP entry ("single", "indel" etc. - see the UCSC genome browser for details).
strand	Strand for dbSNP entry + (or 1) or - (or -1)
snp_alleles	Known SNP alleles, e.g. "AC" (or "A/G") for a SNP of type "single"

Exercise

From genotype to phenotype

- 99.9% of human DNA is identical to another person
- The genotype of an organism is the inherited instructions within the DNA
- A phenotype is any observable characteristic or trait of an organism

Exercise: Genotype to Phenotype

http://wiki.cbs.dtu.dk/teachingmaterials/index.php/ExGenotype2PhenotypeLite